Research Note

Detection of *Campylobacter jejuni* in Various Lymphoid Organs of Broiler Breeder Hens After Oral or Intravaginal Inoculation

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ABSTRACT Two studies were conducted to determine whether *Campylobacter jejuni* could rapidly spread and reside in the internal organs of adult broiler breeder hens. In Study 1, university-housed broiler breeders at 22 wk of age were obtained and placed in individual cages. Each hen was intravaginally inoculated weekly from 23 to 32 wk of age with a characterized strain of *C. jejuni*. At wk 23, 27, and 32, 4 d postinoculation, the hens were euthanized, defeathered, and aseptically opened. In Study 2, university-housed broiler breeder hens were obtained at 42, 53, and 56 wk of age, placed in individual cages, and inoculated either orally or intravaginally with a characterized strain of *C. jejuni*. To reduce the possibility of cross-contamination among samples, the thymus, spleen, liver, and gallbladder were aseptically removed, prior to the ceca. In both studies, all samples were individually analyzed. In Study 1, at 23 wk of age, *C. jejuni* was recovered from 4/7 thymii, 2/7 spleens, 5/7 livers and gallbladders, and 6/7 ceca. At 27 wk of age, *C. jejuni* was recovered from 1/7 thymii and 1/7 ceca. At 32 wk of age, *C. jejuni* was recovered from 4/11 thymii, 1/11 livers and gallbladders, and 2/11 ceca. In Study 2, *C. jejuni* was recovered from 2/6 thymii and 5/6 ceca after oral inoculation and 1/6 spleens, 1/6 livers and gallbladders, and 4/6 ceca after vaginal inoculation of 43-wk-old hens. *Campylobacter jejuni* was recovered from 2/5 thymii, 3/5 spleens, 3/5 livers and gallbladders, and 2/5 ceca after oral inoculation of 53-wk-old hens and 1/5 thymii and 1/5 livers and gallbladders after vaginal inoculation. *Campylobacter jejuni* was recovered from 1/4 thymii, 2/4 livers and gallbladders, and 1/4 ceca and was not detected in any vaginally inoculated birds of 57-wk-old hens. This study provides evidence that *C. jejuni* can reside in the internal organs of broiler breeder hens following oral or intravaginal inoculation.

Key words: *Campylobacter jejuni*, broiler breeder, thymus, liver and gallbladder, spleen

2006 Poultry Science 85:1378–1382

INTRODUCTION

Campylobacter enteritis is a major health problem throughout the world. *Campylobacter*, a gram-negative, microaerophilic bacterium, is believed to be the leading bacterial etiological agent of acute gastroenteritis in the human population. The total number of *Campylobacter* enteritis cases in the United States is estimated at 2.4 million per year, and 80% of the cases are considered foodborne (Blaser and Reller, 1981; Tauxe, 1992; Slutsker et al., 1998). Although the ecology and epidemiology of *Campylobacter* are not clearly understood, many human cases appear to result from ingestion of contaminated water, raw milk, or contaminated foods (Bryan and Doyle, 1995; Finch and Blake, 1995). Mishandling of raw poultry and consumption of inadequately cooked poultry and poultry products are considered to be the primary sources for *Campylobacter*-induced disease in humans (Blaser and Reller, 1981; Park et al., 1981; Kinde, 1990). Studies have found that as much as 80% of processed poultry meat is contaminated with *Campylobacter* (Rosef et al., 1984; Price et al., 2005). The elevated colonization incidence of poultry and subsequent infection in humans has prompted a number of investigations into determining the ecology of this foodborne human pathogen in poultry (Sahin et al., 2002).

One potential mechanism for *Campylobacter* spp. colonization of the bird could be that the bacteria are viable but nonculturable, and dissemination of the organisms via the lymphoid organs of the chicken occurs after hatch. In a study by Cox et al. (2005b), *Campylobacter jejuni* was found to translocate to primary lymphoid organs (thymus and bursa), the spleen (secondary lymphoid organ), the liver and gallbladder, and the ceca of day-old broiler chicks 1 h, 1 d, and 1 wk after both oral and intracloacal inoculations. In that study, a specific sample site did not colonize more often than another site after 1 h postinoculation by either route of inoculation, suggesting that dissemination occurs throughout the

Received December 2, 2005.
Accepted March 16, 2006.
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sites, but what role certain sites play in this movement of the organisms was not determined.

Enterobacteriaceae, streptococci, and lactobacilli have been found to colonize the bursa of Fabricius and the intestinal tract shortly after hatching (Kimura et al., 1986). Bursal lymphocytes have also been shown to migrate to peripheral lymphoid tissue (e.g., spleen; Hemmingsson and Linna, 1972). *Campylobacter jejuni* has been recovered from the lung, liver, and spleen of inoculated quail between 7 and 17 d after oral inoculation (Maruyama and Katsube, 1988). However, whether the dissemination was via the blood or another route was not determined. In addition, it has been shown that *Salmonella* can persist in the spleen for up to 40 wk in chickens (Gast and Beard, 1990; Wigley et al., 2001). *Salmonella* has been found to persist in the liver of orally inoculated laying hens for up to 22 wk postinoculation (Gast and Beard, 1990). The objective of this study was to 1) determine whether *C. jejuni* could disseminate and reside in the internal organs of hens coming into sexual maturity by intravaginal inoculation and 2) determine whether *C. jejuni* could disseminate and reside in the internal organs of hens that have been in sexual maturity after oral or intravaginal inoculation.

MATERIALS AND METHODS

Two studies [1 with young (n = 25) broiler breeder hens and 1 with older (n = 30) broiler breeder hens] were conducted to determine whether *C. jejuni* could reside in the internal organs of adult broiler breeder hens. In Study 1, university-housed broiler breeders at 22 wk of age, which were determined to be negative for *Campylobacter* spp. through fecal droppings, were obtained and placed in individual cages. Each hen was intravaginally inoculated with a 1-mL suspension of a characterized strain of *C. jejuni* at a 10^6 cfu/mL titer weekly from 23 to 32 wk of age. Four days after each inoculation time at wk 23 (n = 7), 27 (n = 7), and 32 (n = 11), hens were euthanized, defeathered, and aseptically opened. In Study 2, university-housed broiler breeder hens, which were determined to be negative for *Campylobacter* spp. from fecal analysis, were obtained at 42 (n = 12), 53 (n = 10), and 57 (n = 8) wk of age, placed in individual cages, and inoculated either orally or intravaginally with a 1-mL suspension of a characterized strain of *C. jejuni* at a titer of 10^6 cfu/mL.

For each trial, broiler breeder hens were held overnight without feed. Euthanasia and processing (up to feather removal) were performed the following morning, and for these experiments, necropsy was limited to removal of the thymus, spleen, liver and gallbladder, and ceca using aseptic techniques. To reduce the possibility of cross-contamination among samples, the thymus, spleen, and liver and gallbladder were aseptically removed first, then the ceca. Individual samples were placed in sterile bags, packed on ice, and transported to the laboratory for evaluation.

The thymus, spleen, liver and gallbladder, and ceca were individually weighed. The samples were then macerated within sterile stomacher bags with a rubber mallet to ensure the contents of the samples were exposed. Bolton's enrichment broth (containing lysed horse blood) was added to the sample bags at a ratio of 3 times the weight of the sample and then stomached (Technar Company, Cincinnati, OH) for 1 min. Samples were incubated in a microaerophilic atmosphere at 42°C for 48 h. A 0.1-mL sample of the enrichment broth from each sample was then plated in duplicate onto *Campylobacter* Cefex agar (Acumedia Manufacturers Inc., Lansing, MI) and incubated in a microaerophilic atmosphere at 42°C for 48 h. Following incubation, plates were observed for presumptive *Campylobacter* colonies. Presumptive colonies were confirmed by microscopic observation of characteristic spiral cells, darting motility in wet mount preparations, and further confirmed through latex agglutination. Isolated colonies were randomly picked and streaked for isolation onto *Campylobacter* Cefex agar and incubated in a microaerophilic atmosphere at 42°C for 48 h. Pure colonies were then placed onto bacterial preservers (treated beads in a cryopreservative fluid) and stored at −80°C. Confirmation utilizing *fla*A sustained virologic response (**SVR**) gene amplification by the PCR method (Hiett et al., 2002) was performed to determine whether the recovered isolates were the same organisms as the characterized strain of *C. jejuni* that was inoculated. All data from the experiments are expressed as the number of positive *Campylobacter* isolates from each sample site over the number of sample sites tested from each hen or as a percentage of the aforementioned.

RESULTS

In Study 1, at 23 wk of age, *C. jejuni* was isolated from 57, 29, 71, and 86% of the thymii, spleens, livers and gallbladders, and ceca, respectively (Table 1). At 27 wk of age, *C. jejuni* was isolated from 14, 0, 0, and 14% of the thymii, spleens, livers and gallbladders, and ceca, respectively (Table 1). At 32 wk of age, *C. jejuni* was isolated from 36, 0, 9, and 18% of the thymii, spleens, livers and gallbladders, and ceca, respectively (Table 1). Overall, *C. jejuni* was isolated from 36, 8, 24, and 36% of the thymii, spleens, livers and gallbladders, and ceca, respectively, after intravaginal inoculation. All isolates saved for characterization were determined to be the characterized strain of *C. jejuni* used for inoculation of the broiler breeder hens based on *fla*A SVR gene amplification by PCR.

In Study 2, *C. jejuni* was isolated from 33% of the thymii and 83% of the ceca after oral inoculation and from 17% of the spleens and livers and gallbladders, and 67% of the ceca after intravaginal inoculation of 43-wk-old hens (Table 2). *Campylobacter jejuni* was isolated from 40% of the thymii and ceca and from 60% of the spleens and livers and gallbladders after oral inoculation of 53-wk-old hens and from 20% of the thymii and livers and gallbladders after intravaginal inoculation (Table

Table 1. Presence of *Campylobacter jejuni* in the primary (thymus) and secondary (spleen) lymphoid organs and the liver and gallbladder and ceca of broiler breeder hens after weekly intravaginal inoculations

	Sample site			
Period[1]	Thymus	Spleen	Liver/ gallbladder[2]	Ceca
Wk 23[3]	4/7[4]	2/7	5/7	6/7
Wk 27	1/7	0/7	0/7	1/7
Wk 32	4/11	0/11	1/11	2/11
Total[5]	9/25	2/25	6/25	9/25

[1]Birds sampled at wk 23, 27, and 32 4 d after intravaginal inoculation.
[2]Combination of the liver and the gallbladder sampled together as one composite sample.
[3]Age of the broiler breeder hens when sampled.
[4]Number of *Campylobacter*-positive samples/total number of samples collected for each week.
[5]Combination of all weeks sampled for each individual sample site.

2). *Campylobacter jejuni* was isolated from 25% of the thymii, livers and gallbladders, and ceca after oral inoculation; however, it was not detected in any intravaginally inoculated 57-wk-old hens (Table 2). Overall, in these older hens, *C. jejuni* was isolated from 33, 20, 27, and 53% of the thymii, spleens, livers and gallbladders, and ceca, respectively, after oral inoculation and from 7, 7, 13, and 27% of the thymii, spleens, livers and gallbladders, and ceca, respectively, after intravaginal inoculation. All isolates saved for characterization were determined to be the characterized strain of *C. jejuni* used for inoculation of the broiler breeder hens based on *fla*A SVR gene amplification by PCR.

DISCUSSION

In recent years, the ecology of *Campylobacter* inside the broiler breeder rooster and hen has been evaluated (Buhr et al., 2002; Cox et al., 2002, 2005a; Hiett et al., 2002, 2003;). In a recent study, *Campylobacter* was naturally isolated from 26% of the mature and 13% of the immature ovarian follicles of broiler breeder hens at different stages in their lay cycle (Cox et al., 2005a). In a study by Buhr and others (2002), *Campylobacter* was recovered from different segments of the reproductive tracts (from 33% of the magnums, 17% of the isthmuses, 58% of the shell glands, 83% of the vaginas, and 100% of the cloacae) of actively laying breeder hens, and it was determined that there was a trend for a greater number of positive samples to occur further down the reproductive tract. In a study by Cox et al. (2002), *Campylobacter* was isolated from 83% of semen samples and 42% of vas deferens sampled from commercial broiler breeder roosters. It has also been shown through use of electron microscopy that *C. jejuni* can apparently attach to chicken spermatozoa (Vizzier-Thaxton et al., 2006).

In a previous study, we found that *C. jejuni* could disseminate to the primary and secondary lymphoid organs of day-of-age chicks 1 h after oral or intracloacal inoculation and still be isolated at 1 d and 1 wk postinoculation from these lymphoid organs (Cox et al., 2005b). In related studies with orally inoculated Japanese quail, *C. jejuni* has been shown to propagate into the spleen, liver, and lungs and persist up to 17 d postinoculation (Maruyama and Katsube, 1988, 1990).

Table 2. Presence of *Campylobacter jejuni* in the primary (thymus) and secondary (spleen) lymphoid organs and the liver and gallbladder and ceca of broiler breeder hens 4 d postinoculation by oral or intravaginal inoculation

	Sample site			
Period[1]	Thymus	Spleen	Liver/ gallbladder[2]	Ceca
Orally inoculated				
Wk 43[3]	2/6[4]	0/6	0/6	5/6
Wk 53	2/5	3/5	3/5	2/5
Wk 57	1/4	0/4	1/4	1/4
Total[5]	5/15	3/15	4/15	8/15
Intravaginally inoculated				
Wk 43	0/6	1/6	1/6	4/6
Wk 53	1/5	0/5	1/5	0/5
Wk 57	0/4	0/4	0/4	0/4
Total	1/15	1/15	2/15	4/15

[1]Necropsy performed 4 d after either route of inoculation.
[2]Combination of the liver and the gallbladder sampled together as one composite sample.
[3]Age of the broiler breeder hens when sampled.
[4]Number of *Campylobacter*-positive samples/Total number of samples.
[5]Combination of all weeks sampled for each individual sample site.

In our study, *C. jejuni* disseminated to and colonized tissues of younger hens after intravaginal inoculation and disseminated to and colonized tissues of older hens after oral and intravaginal inoculation. *Campylobacter jejuni* was isolated from the primary and secondary lymphoid organs sampled and from the liver and gallbladder and ceca from the younger hens after intravaginal inoculation. In addition, *C. jejuni* was isolated from the primary and secondary lymphoid organs sampled and from the liver and gallbladder and ceca from the older hens after both the oral and intravaginal routes of inoculation. Birds of all ages were colonized both in the ceca and in their internal organs. However, 57-wk-old hens were not colonized when the inoculum was introduced vaginally. This could have been due to the organisms not attaching and colonizing the hens; therefore, the organisms may not have entered into the adjacent tissues of the reproductive tract. Also, in older hens, the oral route of inoculation produced a 20% increase in the number of positive isolates compared with the intravaginal route. It is known that fewer organisms of *Salmonella* or *Campylobacter* are needed to colonize the ceca of birds after cloacal inoculation than after oral inoculation due to the harsh environment of the upper gastrointestinal tract. The increase in the number of isolates recovered after oral inoculation vs. intravaginal inoculation could have been due to the organisms having a difficult time traveling up the reproductive tract and into adjacent tissues.

In Experiment 1, the significance of *C. jejuni* disseminating and colonizing the internal tissues of young hens by the intravaginal route was that if a *Campylobacter*-negative hen coming into egg-laying production gets introduced to a contaminated rooster, and the rooster mates with the hen, then the rooster could play an important role in contaminating the hen and causing *Campylobacter* to disseminate to different tissues and reside in these tissues during the laying cycle of the hen. In Experiment 2, the significance of *C. jejuni* disseminating and colonizing the internal tissues of older hens by the oral or intravaginal route of inoculation was that it mimicked contamination via pecking of contaminated droppings or floor material in the scratch area or contaminated semen and showed that either method of introduction could cause *C. jejuni*-negative hens to become positive with the organism and dissemination into internal tissues to occur. However, these results still do not address to what extent oral inoculation, vaginal inoculation, or both play in overall flock contamination. It is evident through this study and our previous study that dissemination of *C. jejuni* into several internal organs of a particular bird occurs after oral, cloacal, and intravaginal inoculation. For the orally and intravaginally inoculated groups, a specific sample site did not colonize significantly more than another site, suggesting that dissemination occurs throughout the sites. What role certain sites play in this movement of the organisms will require additional research. Whether long-term reservoirs are established in breeders and ultimately contribute to broiler flock contamination of *C. jejuni* via these routes has not yet been determined.

ACKNOWLEDGMENTS

We are grateful for the assistance by Debbie Posey, Doug Cosby, and Dianna Bourassa (Russell Research Center, USDA-ARS) for sample collection.

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